Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 51

Project Titles and key words

- Understanding Brain Monoamine Neurotransmission

  Dopamine; Parkinson's disease; Drug addiction

- Brain and Cognition in Function and dysfunction

  Learning, emotion, plasticity, limbic system, animal models

- Dissecting Craniofacial Development

  Cleft lip and palate, Dental abnormalities

- Pathogenesis of *Anaplasma phagocytophilum* in sheep

  Sheep, Anaplasma phagocytophilum, blood, pathogenesis, immunity

- Effect of incubation on post-hatch development

  Avian, incubation, muscle, myofibre, thermal

- Neuropsychological basis of psychiatric disorders

  Mental health, psychiatric disorders, cognition, behaviour

- Dopamine in the pathogenesis of Huntington's disease

  Mouse, triplet repeats, polyglutamine, huntington

- Development of Synthetic Vectors for Gene Therapy

  Gene therapy, synthetic vectors

- Zebrafish model of neurodevelopmental disorders

  Attention, Na+/K+ pump , Laterality, Schizophrenia, dyslexia

- Development of the Nervous System

  neuronal development, cytoskeleton, Alzheimer's disease

Understanding Brain Monoamine Neurotransmission

- Summarise your project (1-2 sentences)

We aim to improve our understanding of the mechanisms through which key brain cells release their chemicals for communication with target brain cells. We hope also to understand some of the processes that go awry in disorders that include Parkinson's disease (PD) and addiction disorders.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Brain cells, or neurons, that use the transmitter dopamine, carry out key functions in our everyday motivated actions as well as out learned habits. We think these neurons tell us about things in our environment that have some motivational value that help us to detect them, and then respond optimally to benefit from them. These same neurons are the targets of addictive drugs, like nicotine and cocaine, which hijack their function and promote the release of the transmitter dopamine. Furthermore, these cells die in the neurodegenerative disease Parkinson's disease. There is therefore a need to understand the workings of these cells better so that we can not only advance biological knowledge, but also improve our understanding and treatment of these kinds of diseases.
The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

- Outline the general project plan.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs behaviour.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

We will raise genetically altered mice that allow us to explore the functions of key molecules in these mechanisms. In some animals we will insert genes into the brain during general anaesthesia which allow animals to express proteins that can be targeted with flashes of light to activate the neural circuit we want to explore. Some animals might instead receive a toxin to make the animals begin to develop a Parkinsonian condition so that we can understand the disease better. Some animals might be given drugs of addiction regularly when awake over a few weeks to enable us to understand better the processes which become disturbed. And a group of animals will have small microelectrodes implanted in their brains and then be allowed to roam freely so that we can understand how neural circuits are important to behaviour.
The adverse effects that some animals might experience might include the effects of brain surgery under general anaesthesia which might include transient pain and bleeding, some disturbances to normal movement, or a failure to thrive.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

This work should therefore advance basic biological knowledge and understanding of

many brain functions relevant to our everyday motivations and actions.
It shed also light on mechanisms relevant to key brain disorders. In turn, we hope to gain insight into potential future therapies for these disorders for which there are currently still very few effective treatments.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

We estimate that we may use up to 3,900 animals in procedures other than simply breeding and maintenance. We may breed and/or maintain up to 19,000 mice, some of which will be the same ones used in the additional procedures.
Mice will be the species used because they are the lowest vertebrates in the phylogenetic tree for which brain dopamine systems are suitably well characterised and comparable to that of humans, as well as there being models for neurodegenerative disease. The mouse is currently the most tractable mammal for use in genetic studies.
We will keep animal numbers to a minimum by using methods and experimental designs that are high yield, allowing multiple measurements per sample, per animal.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

This work is an exploration of fundamental mechanisms of operation of the brain and also studies the adaptive mechanisms and/or the impact of drugs during brain disorders that range from neurodegenerative disease to drug addiction. Use of live animals and real brains is therefore needed to provide tissue with synaptic circuitry that resembles the in vivo scenario. We are not aware of any alternative which does not use animals that would allow progress to be made towards the objective. No cell or culture alternative can adequately provide this.

- Explain why the protocols and the way they are carried out should involve the least suffering.

We will carry out protocols using best practice methods, using monitoring protocols in which we have expertise, and with the earliest endpoints that are commensurate with the scientific aims, in order to ensure suffering is kept to a minimum.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>BRAIN AND COGNITION IN FUNCTION AND DYSFUNCTION</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Learning, emotion, plasticity, limbic system, animal models</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objectives of this project are to understand which bits of the brain are important for different behaviours (e.g. different kinds of learning and memory, anxiety). Having identified which bits of the brain are important we will then work out which neurochemicals are important and what receptors they act on. At the same time we will record brain signals to establish how brain activity changes as animals are behaving in different ways. Having worked out how the brain might work under normal conditions we will then study animal models of diseases such as schizophrenia, Alzheimer’s Disease, Parkinson’s Disease, anxiety disorder and Down Syndrome. We will try and work out why the brain has stopped functioning properly in these different conditions.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Disorders of memory and emotion strike at the very core of our individual selves. The aim of this project is to understand disorders of cognition and emotion resulting from brain dysfunction. As the average age of the population increases, many of these disorders are becoming more widespread. In order to understand how and why the brain might stop working, it is first necessary to understand how the brain works properly under normal conditions. The ultimate goal of the project is to find interventions or treatments or diagnostics that might identify, prevent, alleviate or reverse these brain disorders.</td></tr>
<tr><td></td><td>To do this we must use rats (max. 8200) and mice</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	(max. 15250).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The experiments will involve testing the ability of rats and mice to perform behavioural tasks in which they have to learn and remember crucial information such as where a food reward is or how to escape from a pool of water. We also assess anxiety, for example, by asking the animal whether it wants to explore a new place or remain in a safe location. We will examine the effects of brain lesions, drug treatments and genetic mutations on these behaviours, and record signals of brain activity while the animals perform. The animals will readily learn what to do to get a tasty food reward or how to climb out of the water. Recording of brain signals involves cranial implantation of microelectrodes and could involve single housing of the animals. The expected adverse effects would include brief periods of mild distress during some of the behavioural tests (e.g. after a mild footshock). There may also be transient pain and discomfort after brain surgeries. The experiments are of moderate severity. Animals will be humanely killed at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to show that a particular bit of the brain, or a particular chemical neurotransmitter or receptor, is important for the brain to work properly, it is necessary to remove or silence that bit of brain, or remove or block the neurotransmitter from working. This is not ethical (or practical) in humans. Computer simulations of the brain actually rely on the information that we will provide and so cannot replace the work that we do.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimize the numbers of animals used by making both the behavioural tests and the experimental manipulations (e.g. lesions, genetic modifications) as accurate and sensitive as possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	We work on rats and mice because they are the lowest vertebrate group which reasonably resembles humans. Operations on the brain are done very carefully and in state-of-the-art surgical theatres, and the animals are given pain killers after the operations until they

minimise welfare costs (harms) to the animals.	have fully recovered. Soon after the operations you would not be able to tell the difference between treated animals and controls as they behave in their home cages. It is only on the sophisticated tests of learning and memory that you can begin to tell them apart.

Dissecting Craniofacial Development

- Summarise your project (1-2 sentences)

  Birth defects involving the face and teeth are distressing and represent a global healthcare problem. In this project, we will use mice to increase our understanding of normal facial and tooth development, determine how these developmental events result in facial abnormalities, and test the feasibility of potential treatment strategies.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

  Cleft lip and palate are common birth defects which result from failure of different parts of the face to merge together during development. Each year approximately 1000 babies are born with clefts of the lip and/or palate in the UK alone. Cleft lip and palate can cause major problems with appearance, feeding, speaking, breathing, hearing and social adjustment which can be corrected to varying degrees by surgery, speech therapy, airway support, dental treatment, and psychosocial intervention at major cost to the NHS. In contrast, in developing countries scarce resources put even basic treatment beyond the reach of many thousands of children. Similarly, genetic defects of tooth development are very common and lead to considerable distress.

  As the underlying defects that lead to facial and dental anomalies arise early in embryogenesis and often have complex causes with both genetic and environmental contributions, it has proven difficult to identify causative factors. Although progress has been made in identifying some genetic contributions to cleft lip and palate and disorders of tooth development, the cause of most cases remains unknown. The development of high-throughput sequencing techniques has altered the scale of analysis that can be undertaken providing a unique opportunity to identify the genetic mutations that lead to these distressing birth defects. Although interpretation of human genetic data is difficult, progress will be facilitated greatly by improvements in our knowledge of normal development using mice. By integrating our expertise in high-throughput sequence analysis, developmental biology, computational approaches and human genetics, we will delineate the genetic networks driving development of the lip and palate and apply the information clinically by using it to interpret genetic studies of cleft lip and palate and disorders of tooth development in humans.

- Outline the general project plan.

  This research will be undertaken as a series of four objectives. In Objective 1, we will use new sequencing techniques to generate a complete catalogue of the genes expressed during development of the lip and palate as well as the regions of DNA that control the expression of these genes. In Objective 2, we will refine the predicted genetic networks by analysing the effect on gene expression of disrupting the networks using well-characterised mouse models that are directly relevant to cleft lip and palate in humans. In Objective 3, we will use the data generated in Objectives 1 and 2 to determine the genetic defects that lead to cleft lip and cleft palate in humans. In Objective 4, we will analyse potential treatment strategies for abnormalities of tooth development.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

  The majority of experiments involve breeding of mice that carry similar genetic defects to humans and, as such, do not cause major harm to the animals. Occasionally, animals are injected with a non-injurious substance that allows us to assess cell

behaviour or are fed a diet designed to treat the defect that they display.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

  This research will result in detailed knowledge of the genetic networks driving development of the lip and palate and how they are altered in cleft lip and palate in humans. Determining the genetic mutations underlying cleft lip and palate will have an immediate positive impact as molecular diagnosis and more accurate genetic counselling will become possible for a subset of patients in the short-term. Identification of control sequences will provide a resource of sequences that are targets for understanding the interaction between genetic and environmental contributors to cleft lip and palate in the longer-term. Similarly, testing treatments for abnormalities of dental development in mice will allow us to move towards therapeutic intervention in humans.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

  The mouse is the most appropriate animal for the project. In the case of lip and palate development, the underlying events are almost identical to those occurring in humans. In the case of dental development, the incisor teeth erupt continuously unlike their human counterparts, affording the advantage that all stages of tooth development are represented in an individual tooth leading to reductions in the number of experimental animals used. Importantly, well-characterised mouse models for both cleft lip and palate and tooth development have already been generated. Overall, we estimate that we will use up to 10,000 mice over five years.

  To ensure that the minimum number of animals is used, the highest standards of experimental technique will be maintained thereby reducing the number of experiments that have to be repeated. New members of staff will be trained by experienced researchers to ensure that their operative technique is excellent. All experiments will be planned carefully to ensure that tissue usage is maximised.

  To guard against over-breeding of the mice, laboratory members will keep exemplary breeding records and ensure that the colonies are maintained at the minimum level that will allow successful completion of the experiments.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

  Preliminary data has been generated without the need for animal work; for example, we have utilised human genetic analysis to identify the genetic mutations underlying a number of facial birth defects. These studies have been, and will continue to be, complemented by cell culture biochemical and computational analyses. Nevertheless, when studying congenital malformation syndromes, it is not possible on either logistical or ethical grounds, to perform *in vivo* experiments on human embryos and, as such, the use of animals is the only alternative for research of this nature.

- Explain why the protocols and the way they are carried out should involve the least suffering.

  The majority of our research involves breeding of mice that does not cause suffering to the animals. However, to ensure that all animals are correctly identified so that we use the minimum possible number of animals we may have to take a small piece of tissue from the ear. Animals may experience momentary discomfort associated with this procedure. To minimise transient discomfort, instruments will be maintained in

excellent condition and stress will be reduced by skilled handling.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Pathogenesis of Anaplasma phagocytophilum in sheep</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Sheep, Anaplasma phagocytophilum, blood, pathogenesis, immunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Research under the current project will investigate the mechanisms of infection and persistence of the tick-borne pathogen Anaplasma phagocytophilum in its natural mammalian host. While it can infect a wide range of hosts, including human beings, sheep, goats and cattle appear to be the most susceptible hosts. In the United Kingdom and other parts of Europe, hundreds of thousands of lambs are reported to die each year as a result of infection with A. phagocytophilum and complications with secondary infections such as tick pyaemia. It is also responsible for severe losses in the dairy industry due to reduced milk yield, abortions and secondary infections. Therefore there is a good welfare and economic justification to use a few animals to investigate the mechanism of the disease process of this unique organism. This will be important for the development of vaccines to protect sheep, cattle and other animals from infections. Furthermore the present study will help to clarify some of the mechanisms by which this organism manages to selectively infect and multiply within the neutrophils, which are at the forefront of the body’s immune defence system.
Although methods to grow the organism in vitro have now been developed, the only way to investigate the disease process is by using the natural hosts (sheep, goats or cattle) or susceptible laboratory animals. By using the natural hosts we hope to reproduce condition close to the disease that affects sheep, goats and cattle. Most of the work will use laboratory-based molecular and antigenic studies, using bacteria derived from infected cell cultures. Where replacement of animals is not possible we will use the smallest number possible and the least invasive methods. Refinement will include running pilot</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	experiments using a small number of animals to establish optimal methods of detection and identification of bacteria and specific cell types. The project aims to improve the welfare and productivity of domestic farm animals by investigating the disease processes of this unique tick-borne agent. The results of the study will provide important information about the disease and will not only improve the well-being of animals but it will also help to reduce the possibility of human infections acquired from animals. It will also add new insights into the ways by which some bacterial pathogens evade the body's immune system and the effects of such agents of the immune system.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	*Anaplasma phagocytophilum* is an important cause of disease in sheep and cattle in tick-infested pastures of the UK and other parts of Europe. It is also recognised as an emerging human pathogen. Therefore in addition to the animal welfare and human health benefits by investigating the mechanisms of pathogenesis and immunity, the present project will also help to advance knowledge regarding the survival of this unique organism in the hostile environment of the neutrophil.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 156 sheep
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main adverse effects will be a self-limiting febrile reaction lasting a few days, and occasional development of haematoma in the area of injection and withdrawal of blood from blood veins. The expected severity will be mild to moderate At the end of the experiment, the animals will be either killed under Schedule 1, or returned to Leahurst Animal Farm, after treatment with antibiotics.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	After years of research, the strains of *A. phagocytophilum* to be sued in the present study can now be grown in cell culture systems derived from its tick host. This will allow us to generate large volumes of bacteria to prepare antigens for immunological studies and also to generate bacteria to be used to study the effects of the agent of immune cells derived from uninfected healthy animals. However, as the main aim of the project is to understand the mechanism of infection, immune responses and persistence, it would be necessary to use the most susceptible natural hosts (sheep). To reduce the number of animals used, cell-culture derived bacteria and cell lines

	which functions as neutrophils and monocytes (e.g. HL60 and THP1 cell lines) will be used to standardize laboratory methods to assay the effects of *A. phagocytophilum* on some of their function. Whenever possible, cell culture and molecular and other laboratory methods will be used for assays which do not require live animals. Also when animals are used the least number of animals necessary for statistical validation will be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous studies have shown that sheep kept under tick-free conditions are extremely susceptible to *A. phagocytophilum*. As all the animals to be used in the present project will be raised and reared under tick-free conditions, we will only use the minimum number required for statistical analysis. The number of animals to be used will be kept at absolute minimum necessary for statistical analysis and validation of the data. The estimated number of sheep to be used over a period of 5 years is 80. In all cases the minimum number of animals needed will be estimated using well-established statistical power curves.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Although the organism has recently been cultivated *in vitro*, there is a need to use a small number of its natural hosts (sheep), to investigate its pathogenesis in general and its ability to evade the immune system in general. We need to use sheep because the strains which infect ruminants do not infect laboratory animals. By using the natural hosts, we hope to reproduce conditions close to the disease that affects sheep. The other advantage of using sheep instead of laboratory animals is that we will be able to take sequential blood samples from the animals without causing too much discomfort or pain over a longer time frame. Studies over the last decades have shown that sheep bred and maintained in tick-free conditions are extremely susceptible to infection with *A. phagocytophilum*, the causative agent of tick-borne fever, thus allowing us to reduce the number of animals to be used. The planned experiments are not likely to cause serious adverse effect but all animals will be monitored closely for the development of any adverse effects and treated appropriately

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Effect of incubation on post-hatch development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Avian, incubation, muscle, myofibre, thermal</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>1</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td><s>Yes</s></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this project is to test the hypothesis that relatively small and short increases in incubation temperature have the ability to increase breast muscle fibre mass and that the effect is due as much, if not predominantly, to an increase in the number of fibres rather than an increase in the mass of individual fibres. By examining the period in embryogenesis when muscle fibre number is determined it is anticipated the necessary information to understand the relationship between thermal manipulations and hyperplasia will be obtained. It is also intended that the study will provide important information on so-called lean growth, lean growth being determined in part by prenatal increases in muscle fibre number.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The benefits arising from this work include the advancement of our understanding of muscle biology and, for the poultry sector, provides a better understanding of the implications of temperature manipulation, either desired or inadvertent (as can happen in the centre of a stack of eggs when being incubated) during incubation.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Gallus Domesticus, 1000 animals, 52 weeks</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>No adverse effects are expected due to the thermal manipulations. Hatchability and liveability shouldn’t be negatively impacted. Animals remaining at the end of the trial will be euthanized humanely.</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

level of severity? What will happen to the animals at the end?	
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The test variable affects the development of the whole animal. Therefore a cell culture cannot be used to test the effect of thermal manipulations on muscle growth.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The variability of breast yield was assessed and used to calculate the necessary number of birds to obtain a valid measurement. No additional birds will be utilized.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Chickens are an ideal species due to small size, low upkeep and previous research in this field have been done using chickens. The model was chosen as to minimize variability from effects such as hatch, batch or other blocking effects. The method of obtaining breast yield is a standard procedure, but coupled with histology offers validation to the effectiveness of making selections on early breast yield. While the measurements needed are destructive, birds will be euthanized humanely prior to any data collection thus minimizing stress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Neuropsychological basis of psychiatric disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Mental health, psychiatric disorders, cognition, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td><s>Yes</s></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Our research aims to understand how and why neuropsychiatric disorders occur, and to develop new treatments for people suffering from these disorders. Building on our previous work, this research aims to identify new drug targets and new forms of behavioural therapy that could treat neuropsychiatric disorders, and with further refinement and understanding provided by our work with animal models, these new therapeutic avenues should soon be ready for translation into human studies.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Mental health problems cost the UK an estimated £77 billion per year. Thus, neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD), post-traumatic stress disorder (PTSD) and drug addiction, place a considerable burden on not only the affected individual, but also social and economic burdens on society. Our research aims to understand the bases of these disorders, and develop new treatments for them. We use rodent models to define the psychological, neurobiological and neurochemical bases of different neuropsychiatric disorders, to investigate why certain subpopulations are vulnerable to neuropsychiatric disorders, and why they respond differently to treatment.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">We use rodent behavioural models (both rats and mice). We use the minimum numbers of animals possible to achieve biologically and statistically meaningful data. We anticipate that we will use fewer than 10420 rats and 1320 mice over 5 years.</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the majority of our animals, we anticipate no more than transient discomfort and no lasting harm. However, as some of the disorders we study cause distress in humans (e.g. post-traumatic stress disorder, or drug addiction) then some of our animals do experience more stressful conditions (of no more than moderate severity). In some of our experiments, we have to manipulate the brain (e.g. by surgically damaging specific regions) in order to understand why neuropsychiatric disorders happen. When we need to do either of these, the animals are very carefully monitored for any signs of pain or distress. If the animals show signs of suffering and we are not able to ameliorate these in consultation with the named veterinary surgeon, then we euthanize the animal. Fortunately, such instances are very rare. At the end of experiments, the animals are killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is only possible with the use of animals. Human studies (e.g. brain imaging studies) are useful, but can only provide correlative data that do not address causation. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie predisposition to, and the development of, neuropsychiatric disorders in humans. Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models first. *In vitro* models (e.g. brain slice preparations) or computer simulations cannot be used because the modelling of behaviour in these systems is not sufficiently advanced.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use rats and mice because they are the least sentient species that can model neuropsychiatric disorders. The brain circuitry implicated in many neuropsychiatric disorders is highly conserved between rodents and humans, and the behavioural tasks that we have developed are widely recognised as modelling specific aspects of these disorders. We take the welfare of the animals very seriously. Most of our animals run in long-lasting behavioural experiments in which they perform tasks for food reward, and experience procedures, e.g. injections, that produce only transient discomfort and no lasting harm. Animals are monitored frequently (often undergoing daily testing) and any adverse effects are discussed with

	the named veterinary surgeon. If these cannot be quickly ameliorated then animals are euthanized to prevent suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="4">Dopamine in the pathogenesis of Huntington’s disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="4">Mouse, triplet repeats, polyglutamine, huntingtin</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="4">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td colspan="4"><table>
<tr><td>Basic research</td><td>Yes</td><td></td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td>No</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="4">Huntington’s disease is caused by a genetic mutation in a single gene that produces a toxic protein. Humans develop symptoms in their 3rd to 5th decade of life; these symptoms are progressive and invariably fatal. Currently there is no cure and therapy is limited to treating symptoms. There is a growing awareness that the brains of people with the mutation are affected long before the onset of overt symptoms. By understanding these early changes, it may be possible to identify drug targets to slow or halt the progression of the disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science</td><td colspan="4">This project will focus on the early changes in the properties of brain cells</td></tr>
</table>

9 Delete Yes or No as appropriate.

10 At least one additional purpose must be selected with this option.

could be advanced or humans or animals could benefit from the project)?	that release dopamine. Under certain circumstances dopamine is also toxic and it is possible that altered dopamine function could enhance the toxicity of the mutant protein, triggering further toxic and damaging changes in the brains of patients. By understanding how dopamine releasing cells are affected in early disease we will be able to develop strategies to halt or reverse dopamine-related toxicity in Huntington's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Brain tissue will be harvested from mice that carry the human Huntington's disease gene. Over the five year period of this licence, we expect to use about 200 mice per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The mice carry a version of the human mutation that confers a mild form of Huntington's disease. They do not show overt symptoms until about a year of age and have a near-normal life-span. Animals will be humanely killed and their brains used to investigate dopaminergic function. Animals that show signs of developing moderate symptoms (such >20% loss of body mass) will be humanely killed and their brains banked for later analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because of the complex nature of the cellular interactions it is not possible to model Huntington's disease using cell culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A statistical test, called power analysis, will be used to ensure no more animals than absolutely necessary are used to determine the outcome of an experiment. This will determine the size of the breeding colony so only the numbers required for the studies carried out under this licence will be produced.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Because we are focussing on the early changes in Huntington's disease we have chosen not to use mouse models that exhibit rapidly progressing and severe forms of the disease. Instead, we will use a mouse model that carries a transgene conferring a mild form.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Development of Synthetic Vectors for Gene Therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Gene therapy, synthetic vectors</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The scientific basis and justification for this project is in pioneering the translation of nanotechnology to preclinical gene therapy. Our focus is to perform basic chemical biology and nanotechnology research, and then transform knowledge gained into successful new therapies with a specific interest in genetic therapies.

The overall goal of this project is to generate new gene delivery systems for efficient gene transfer in vivo, with therapeutic efficacy in varied disease models.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Despite initial promise and substantial progress in gene therapy research, human gene therapy is still based on technologies that so far do not allow for routine clinical use. Safety and efficacy in gene therapy depend upon selectively delivering the therapeutic gene to the correct tissue without inducing harmful side effects. Due to complications associated with immunity, toxicity, limitations in dose, and difficulties in scaling operations for mass clinical production, viral vectors do not offer the best qualities for use in clinical gene therapy. By focusing on the development of synthetic and hybrid vectors with obvious potential for large-scale production, we hope to see the technology developed in our lab applied on a mass scale in a clinical setting for the treatment of any number of genetic and acquired diseases in man. Therefore we expect that the benefits of this research to be substantial as we have demonstrated the benefits of using viral and in particular non-viral vectors for the treatment of genetic diseases including</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

	atherosclerosis, cancer, stroke and Parkinson's disease, and hopefully for musculoskeletal diseases too. The prevalence of all of these diseases is rapidly increasing worldwide and therefore seeking new effective therapeutics is of extreme importance. In particular, degenerative diseases such as Parkinson's disease and musculoskeletal conditions are beginning to have a huge socioeconomic impact in the Western world due to the largely aging populations. Therefore the development of effective therapeutics would not only benefit patients on an individual day-to-day basis but also at a higher level both socially and economically. Despite the high cost of research and development of these types of therapeutics, it is of upmost interest to the public and society as a whole, and would in the long term have an impact as an economical necessity.
What species and approximate numbers of animals do you expect to use over what period of time?	Both mice and rats will be used to achieve our 5 year project objectives. In total to achieve our 9 project objectives, 18,000 animals will be used (more specifically 14,000 mice and 400 rats).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Preliminary experiments to determine the biodistribution, biological activity, therapeutic potential and safety will be performed in healthy animals. Further experiments will be performed in diseased animals to explore the therapeutic potential of these new gene therapies. Different disease models will be used to explore potential new gene therapies, including cancer, atherosclerosis, Parkinson's disease and skeletal muscle injury. All of these disease models are of moderate severity. Animals will experience some discomfort, distress or pain, however all steps will be taken to minimise these adverse effects, including compulsory pain relief. The earliest end-points will be chosen to cull the animals in order to minimise any actual/potential suffering to the animal. Humane methods of killing will be used.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is an unfortunate truth of non-viral vector mediated gene therapy that *in vitro* data does not shed much light on the *in vivo* performance of a specific vector. However, much of the previous work in synthetic vector development has focused on transfecting cells *in vitro*. There have been very few thorough studies characterizing new vector functions *in vivo*, leaving little chance for any clinical study using most of these vectors. While we will make every effort to study *in vitro* the efficiency of gene expression, which depends upon the escape of the nucleic acid within the synthetic

	vector from the endosome into the cytosol after the vector has entered the target cell, it will still be necessary to study the uptake and distribution of these vectors, as well as their immunogenicity, in animals. Biodistribution and gene expression are complex outcomes that depend on interaction of the vector with blood, the immune system, and multiple tissues. No *in vitro* systems come close to this kind of complexity. Biodistribution, gene transfer efficiency, and long term gene expression studies could theoretically be conducted in numerous small animal models. Mice are chosen for this study for a variety of reasons, primarily as they are the least sentient species that give data applicable to human clinical studies. Additionally, the mouse is used for numerous gene therapy studies as its response to a variety of traditional gene transfer vectors and reporter genes is well characterized. Immunocompromised mice are sometimes required when human tumour cells are used. More specifically for tumour studies, tumour growth (flank or metastatic) is dependent on several features of the living animal, e.g. tumour/host stroma interactions, angiogenesis, organ micro-environment, etc., which cannot be fully replicated *in vitro*. Therefore, in order to translate findings towards the clinic, *in vivo* modelling is essential. Numbers of animals to be used will be minimized by thorough evaluation of each proposed vector *in vitro* where feasible and/or appropriate, and by engaging in stepwise experiments – if a vector fails to show promise in early studies, it will be dropped from further *in vivo* characterization until it has been reformulated to improve performance. By running experiments in parallel with well characterized vectors, control groups can be used for multiple experiments. In terms of *in vivo* Parkinson's disease modelling, both mice and rats can be used, although rats are the preferred animal model since the 6-OHDA induced hemiparkinsonian rat model is the most well-established. Some *in vitro* Parkinsonian models have been established although they do not truly reflect the complexity of the disease and have many limitations. Therefore, rodent models of Parkinson's disease are essential in assessing *in vivo* efficacy of gene therapy vectors. Furthermore, behavioural testing can be performed in rodents to assess therapeutic efficacy of gene therapy vectors; a crucial read-out that cannot be obtained from *in vitro* modelling. Each experimental group will consist of 6 animals which have been

	consistently found to be an effective minimum for statistical analysis of data resolution of significant results. A crucial factor which will be considered is the data/animal ratio which is greatly dependent on the variables assayed for *ex vivo.* These will be as extensive as possible and samples may be fixed or frozen to enable subsequent assays in the future. Similarly, for the rodent skeletal muscle injury model there are no suitable alternative *in vitro* models available and functional/behavioural testing will be performed to assess therapeutic efficacy of gene therapy vectors at regenerating injured skeletal muscle. Therefore *in vivo* modelling is essential for this purpose, although animal numbers will be minimized where possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All steps will be taken to minimise animal use and to obtain as much information from fewer animals, thereby reducing the future use of animals. One major area where we will reduce the number of small animals used is by using live non-invasive, whole body imaging. Techniques that will be applied are X-ray, CT, SPECT, PET, MRI, MSOT and IVIS imaging which will all help to reduce the number of animals used in basic/translational research. The same animal will be imaged multiple times in order to monitor visually, usually in real time, the progression/regression of disease (e.g. tumour growth rate) or to track the biodistribution and residence time of gene therapy vectors both in healthy and diseased animals. Utilising imaging techniques will help avoid the need to sequentially sacrifice animals at different time points, ultimately allowing for significant reductions in the number of animals used per study. Furthermore, improved experimental design and statistical analysis will allow for further animal number reduction. More specifically, by choosing a suitable experiment design (e.g.: completely randomised, randomised block, Latin square, etc.) and deciding on the statistical analysis before starting the experiment can allow for further reduction of animal numbers, bearing in mind that statistical methods may be modified when the results are obtained.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For cancer related studies, flank tumours will be considered our first-line model, when possible, in order to minimise the need for the metastatic type and its associated adverse effects. Both targeted and non-targeted vectors will be used here. Metastatic tumour models in immunocompetent mice will be used for testing targeted vectors with long circulation time (shown from flank models) in order to test their ability to reach the tumour site

	and achieve the required therapeutic effect. Metastatic type will be used as a first choice only when tissue characteristic (i.e. lung or liver) is a prerequisite for the therapeutic mechanism (i.e. bearing specific receptors in targeted delivery). Animals will be culled before the development of severe side effects. Toxin-induced models of Parkinson's disease (6-OHDA and LPS) will be used to assess the therapeutic efficacy of novel gene therapy vectors for modifying the disease process and the ability to offer neuroprotection. The 6-OHDA induced hemiparkinsonism model is the most well characterised and reproducible model of Parkinson's disease in rodents, both at the pathological and functional (behavioural) level. Similarly, LPS-induced parkinsonism is also well characterised, particularly in mice. The reproducibility and consistency of these moderate severity models of Parkinson's disease will enable us to reduce the number of animals needed per study but also improve the quality of data obtained both at the biological and behavioural levels. Both the models of atherosclerosis and skeletal muscle injury will be achieved by performing either chemical or mechanical injury to the blood vessel or muscle, respectively. Pilot studies will be performed for both disease models to determine by which means (either chemical or mechanical insult) achieves the most reproducible pathology and symptoms of disease but also with the least suffering and distress caused to the animal (including mortality rate). The refinement of all scientific procedures and maintaining a high-level of husbandry will help minimise the actual/potential pain, suffering, distress, or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Refinement of all procedures will not only benefit the animals but will also improve the quality of our findings, increasing reproducibility and reliability of all results. First of all, non-invasive techniques will be used where possible/suitable, for example: choosing the least painful and stressful route of administration of gene therapy vectors, considering the volume and frequency of administration too. Wherever necessary appropriate anaesthesia will be employed to further reduce the suffering and distress caused to experimental animals. Appropriate analgesic will always be used for pain relief and for all protocols

	the earliest endpoints will be used where possible. After each procedure is performed, animals will be closely monitored for adverse effects (mild, moderate, severe) and appropriate steps will be taken to minimise suffering, pain or distress. For example, shortly after inducing Parkinson's disease, sometimes the rats are less mobile and are unable to reach food in the hopper of the cage, therefore food pellets and hydrogels are placed inside the cage to prevent disruption to feeding, weight loss or dehydration. In most cases approved Schedule I procedures will be used to cull animals, however, on occasions perfusion and fixation procedures may need to be performed in order to preserve the tissues for post-mortem analysis. On such occasions, animals will be given a suitable dose of non-recovery anaesthesia in order to minimise the suffering/ pain felt. Before any procedure is performed, all animals will be handled in order to familiarise them with the researcher, thus allowing for voluntary co-operation with procedures like blood sampling and behavioural testing. This will allow the researcher to have greater control over the procedure and both the handler and animal will be less stressed, plus better quality data will be achieved. Furthermore, by maintaining clean and spacious accommodation for the animals, and enriching their environment so that it meets the animals' physical and behavioural needs, for example: providing nesting opportunities for rodents or the opportunity to climb and hide, suffering and distress caused to the animals will be reduced.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Zebrafish model of neurodevelopmental disorders.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Attention, Na^+/K^+ pump , Laterality, Schizophrenia, dyslexia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>✓Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>✓Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Summary: The aims of this project are to understand how brain networks develop and support complex cognition and how this function is impaired – and potentially repaired – in psychiatric and neurological disease.
The project will explore brain structures and genetics underlying cognition. Impaired cognition is common in many developmental and psychiatric conditions, including schizophrenia and dyslexia. Genetic mapping has identified candidate genes for both of these disorders. This project is primarily concerned with ‘translational’ research – basic research in animals that aims to improve understanding of mechanism. With limited treatments available for neurodevelopmental disorders, we need to use model in vivo systems that provide data which can potentially predict clinical utility. Zebrafish are an increasingly being used in this way as they breed readily and genetic lines are available with changes in the relevant candidate genes.
By the conclusion of the project, we anticipate that we will have achieved a greater understanding of brain organisation mediating the control of complex cognition.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Investigating the molecular mechanisms underlying genetic associations is essential to understand the underlying biology and develop better tools that will have clinical relevance (early detection, diagnosis, intervention).
This is an opportune moment to study the neural</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

	basis of human disorders in zebrafish, with a view to identifying new treatment strategies for psychiatric and neurological conditions. There is a very realistic chance of a treatment breakthrough as a result of improved pre-clinical models, with validation by cognitive assessment. A benefit that could result from this project would be progressing a novel compound to clinical trials for the treatment of psychiatric illness, developmental and motor disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use ~750 zebrafish. The number is based on previous research, and with assumptions about the likely maximum number of personnel working on experiments within the licensed project (estimated to be 1 post-graduate student and 2-3 undergraduate students per annum). In practice, the number of animals for each individual experiment is determined statistically as the minimum number that will permit scientifically-acceptable counterbalancing of conditions. Parallel work in cell models will help defining hypothesis to test reducing the number of animals involved in the screenings.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures (including, single housing & drug administration) are considered mild; animals are not expected to suffer as a result of the treatment. Neural interventions (administering drugs) are given at doses that are consistent with testing in a behavioural task; therefore serious adverse reactions (defined as rendering the animal not able to be tested) are neither intended nor expected. Animals will be monitored each day for signs of ill health and any animals showing signs of stress or toxicity are removed. Our genetic manipulation will target regions of the genome regulating genes activity to assess at which time point and in which tissues they might be relevant for neurodevelopment. Therefore, because we are not disrupting the function of any gene (as in a knock-out experiments) we will only expect subtle effects at phenotypic level. Care will be taken to ensure any fish exposed to drug treatments, exhibiting an acute avoidance response will be removed into a drug free environment and allowed to recover. Chemicals that cause obvious signs of distress will not be continued. Any fish showing signs of adverse effects will be killed by schedule 1 procedure.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The objective is to assess the cognitive deficits in pre-clinical animal models, so this can only be done in the model itself – animals must be used. The measure of interest is covert cognitions (which cannot be seen) that are reflected in overt behaviour (which is the measure of interest): therefore, it is not possible to study them in anything other than a conscious, behaving animal. It is well established that animal models are necessary to understand function of genes relevant to human diseases.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always try to minimise the absolute number of animals required. For example, the experiments are designed so that, wherever possible, the performance of an individual animal is taken at 'baseline' and changes are observed according to the treatment administered. By comparing changes in behaviour in each animal (as opposed to comparing groups of animals who receive different treatments), not only is the absolute number of animals used kept to a minimum, but the ability to detect changes as a result of the treatment is considerably enhanced. We will conduct statistical power tests to calculate the fewest number of animals required in order to achieve our objectives as stated. Gene knockdown experiments are qualitative in nature assessing structural and morphological changes following genetic manipulation. Once the experimental procedure will be fully established (work conducted mainly at embryonic stage) only few replicates to validate the results will be necessary. We will use well established methodology applying existing protocols which will not require extensive optimisation. The work conducted in the cell lines model will guide the hypothesis we will test limiting the number of animals we will sacrifice for immune-histochemistry and imaging techniques. We have readily available support from collaborators who have already conducted these types of studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In the past, by collaborating with scientists in various companies (GSK, Organon, Shering-Plough; Lundbeck; Wyeth) we have ensured that our work is directed to the front line of clinical need (as perceived by market demand for treatments) and operates according to best practice in the pharmaceutical industry as well as in the scientific literature. As the study of complex behaviour is not possible without the use of animals, care is taken to minimize suffering where possible. Within our

	facility we have 2 experienced technicians, who regularly monitor the health of the fish and 2 fully trained NACWOs on hand to provide knowledge and care of the animals. Zebrafish have now proved to be a very powerful biological model to study neurodevelopment and understand the function of many genes underlying human diseases. The main advantages of zebrafish are: effective low maintenance costs, a rapid life cycle and with external embryonic development and simple optical visualization. Most importantly it has already proved to be an extremely powerful tool to understand human diseases and several genetic technologies have now been developed for gene manipulation. With this proposal we will study specific genetic variants affecting gene expression regulation rather than more penetrant mutation affecting directly gene function. Therefore we do not expect the transgenic procedure to lead to significant suffering. However we will require assessment of the phenotype through a series of imaging techniques requiring sacrificing the animals. In each case we will use MS222 or other appropriate anaesthetic to ameliorate animal suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development of the Nervous System</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>neuronal development, cytoskeleton, Alzheimer’s disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The main objective is to understand, at a molecular level, how developing neurons capitalise on the dynamic properties of their cytoskeletons to achieve various cellular processes such as forming a neurite, growth cone pathfinding and synaptic plasticity.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The project will make a contribution to our understanding of how the nervous system develops in the embryo and how synapses function to form memories. This has several practical consequences because many nervous system illnesses such as autism and Schizophrenia are neuro-developmental disorders and in Alzheimer’s disease, there is a malfunction in the formation of new memories because of a loss of a key cytoskeletal protein from synapses.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse and rat, 1,000 over 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The experiments begin with the death of the animal since we are obtaining tissue from embryos or neonates to either grow in culture or examine biochemically.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] At least one additional purpose must be selected with this option.

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals as a source of neuronal cells for our cultures because there are no immortalised cell lines that can substitute for primary neurons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We rarely need to use an entire litter for our experiments and so we have established a network of scientists who also culture neurons to share litters with.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents have a long and extensive history of use in the field of neuronal development and a great deal is known about the development of their nervous systems. The mouse is the closest experimental animal model with tractable genetics to humans that we have.